J. Mycopathol. Res. 29(2) : 149-154 (1991), Printed in India.

Standarization of Inoculation Method and Screening of Different *Citrus* Species Against Powdery Mildew Disease

SAMIT DUTTA ROY, N. MUKHERJEE AND S. MUKHOPADHYAY

Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, West Bengal 741235

Inoculation methods for the powdery mildew disease of *Citrus* caused by *Oidium tingitaninum* Carter were standarized. Touching and dusting of spore on leaves under humid condition were the most effective methods.

Twelve species of *Citrus* and *Poncirus trifoliata* member of the family Rutaceae were tested for their resistance against powdery mildew. No genus or species was absolutely resistant, but showed different degree of susceptibility. Stomatal number and size, number of oil gland and thickness of the cuticle and epidermal layer did not have any clear relation with degree of susceptibility to powdery mildew.

Key words : Powdery mildew, *Citrus* species, *Oidium tingitaninum* Carter

INTRODUCTION

Powdery mildew disease is very common in orange growing areas. The disease is characterized by a white powdery growth on different surfaces of the plant, specially on the young actively growing twigs. Infected leaves become yellow, dry up and fall off resulting in dying back of the branches. Due to this disease the crop suffers from a heavy loss in yield (Devarajan, 1946 and Dutta Roy *et al.*, 1986).

Many workers screened different *Citrus* species against powdry mildew and they found none of the species to be immune, but according to them different species exhibited varying degrees of resistance (Ullasa and Naidu, 1975 ; Raghavendra *et al.* 1977 ; Ram *et al.* 1977 ; Reddy *et al.*, 1984 and N. rayanappa and Ravishankar, 1985, 1986). The present work deals with the standarization of inoculation methods and screening of the different species of Rutaceae against powder

mildew disease. Attempts have been made to compare the co-relation of powdery mildew disease with the different anatomical features of the host leaves.

MATERIALS AND METHODS

Two years old potted Darjeeling orange plants (*Citrus reticulata* Blanco) were used for artificial inoculation Three month old leaves were inoculated by four methods

(i) Direct spore touch—Infected leaves were directly brought intouch with the moist healthy leaves

(ii) Spore dusting—Freshly collected spores were dusted on the moist healthy leaves

(iii) Spore rubbing—Diseased leaves carrying spores on their surface were rubbed on the moist, healthy leaves

(iv) Spraying water suspension of the freshly collected spores (40000 to 45000 no/ml) were sprayed on healthy leaves with the help of throat sprayer. Inoculated plants were covered with glass chimney and the rates of disease development were recorded using 0 to 4 scale by different days after inoculation (Dutta Roy, 1990).

Different species of *Citrus* and *Poncirus* have been selected for screening against powdery mildew. Seedlings of these were raised in earthen pots and allowed to attain the age of 1 year. One set of plants were then inoculated with respective powdery mildew spores by dusting method described earlier. In a other set of experiment the plants were inoculated with the powdery mildew spores from *C. reticulata.* Intensities of powdery mildew disease on both sets of plant after different days were recorded.

In order to examine the possibility of any relationship of the anatomical characteristics of the leaves (stomata and oil gland) of *C. reticulata* (var. Darjeeling orange - a susceptible species) and *Poncirus trifoliata* (a resistant species). Small pieces (sq. mm each) of lamina from different regions were taken for the study. Dermal preparation was done by oxidising the material with Schultze's reagent (Sass, 1951).

In order to study the relationship between epidermal layer with powdery mildew disease incidence, cross-section of the healthy leaves of the above two species were prepared. The cross-sections were passed through different grades of alcohol for dehydration and finally mounted in DPX, sor microscopic observation and measurement.

RESULTS AND DISCUSSION

Methods of inoculation

Among the four methods spore-touching and spore dusting methods appeared to be best and the symptoms were appeared on leaves after different days of interval. Disease intensity became 80 percent aftet 11-12 days. Spraying methed failed to produce any symptoms (Table 1). Rubbing method, however, was able to produce disease symptoms though not to rhe extent that could be done by touching and dusting method.

Table 1. Efficacy of four methods of artificial inoculation for powdery mildew diseese on Darjeeling orange plant

Methods	Disease intensity after days of inoculation						
	1	3	5	7	9	11	12
Spore touching	0.0	17.0	25.0	64.0	76.0	80.0	80.0
Spore dusting	0 0	13.0	18.0	60.0	76.0	80.0	80.0
Rubbing	0.0	0.0	5.0	13.0	13.0	25.0	25.0
Spraying	0.0	0.0	0.0	0.0	0.0	0.0	0.0

'0' — no symptom

Table 2. Reaction of different genera of Rutaceae family towards powdery mildew

Host genera	Inoculated source	
	Same host	Different host
Darjeeling orange (*C. reticulata* Blanco), Cleopetra orange (*C. rashni* Tanaka)	80*	80*
Sweet orange (*C. sinensis* (Linn.) Osbeck) Sour orange (*C. aurantium* Linn) Kagziline (*C. aurantifolia* (Christm.) Swingle) Rough lemon (*C. jambhiri* Lush), Rangpur lime (*C. limonia* Osbeck)	60*	60*
Pummelo (*C. grandis* (Linn.) Osbeck), Grape fruit (*C. paradisi* Macf)	25*	25*
Gajanima (*C. pennivesiculata* Tanaka), Carrijo (*C sinesis* (Linn.) x *C. trifoliata* (Linn) Raf.), Citron (*C medica* (Linn.), Trifoliata (*Poncirus* trifoliata Raf.)	5*	5*

* Average of 10 replicates
Data collected 192 hours after inoculation

Varietal screening

All the *Citrus* species tested produced the powdery mildew disease although the degree of susceptibility between the species varied greatly. Species like *C. pennivesiculata* Tanaka and *C. medica* Linn. were affected very little with the disease, but on the other hand *C. reticulata* Blanco and *C. reshni* Tanaka were most susceptible against this disease (Table 2). From the cross inoculation studies, it appeared that the powdery mildew fungus occurring on these different *Citrus* species in this area might be the same.

Relationship of leaf character with resistance

From the results of anatomical studies of different host leaves in the Rutaceae (Table 3), it was found that the number of stomata in the upper surface of the leaves were very few in number (negligible) compared to those in lower surface Again with regard to the number of stomata in the young leaves, it was higher than those in older leaves.

Table 3. Stomatal and Oil gland number and size in varieties susceptible and resistant to powdery mildew

Host	Young leaves						Old leaves					
	Upper Surface			Lower Surface			Upper Surface			Lower Surface		
	No. (per mm_2)	L (μm)	B (μm)	No. (per mm_2)	L (μm)	B (μm)	No. (per mm_2)	L (μm)	B (μm)	No. (per mm_2)	L (μm)	B (μm)
STOMATA												
Darjeeling orange	—	—	—	755.9	2.5 to 5.0	1.5 to 2.5	—	—	—	224.8	7.5 to 12.5	5.0 to 7.5
Trifoliate	—	—	—	259 6	7.5 to 20 0	5 0 to 10.0	—	—	—	246.3	7.5 to 20.0	5.0 to 10.0
OIL GLAND												
Darjeeling orange	5.0	86.0 to 103.0	86.0	2.7	68 0 to 86.0	68.0 to 86.0	4.8	86.0 to 120.0	68.0 to 103 0	3.0	86.0	68.0 to 86 0
Trifoliate	3.0	120.0 to 189.2	120 0 to 172.0	3.3	86.0 to 120 0	68.0 to 86.0	3.4	68 0 to 86 0	68.0 to 86.0	1.7	68.0 to 86 0	68.0 to 86.0

"—", Very few in number ; L, Length ; B, Breadth ;

Between the susceptible and resistant genera stomatal number and size did not vary significantly and meaningfully. In fact it seemed to have no correlation with the susceptibility of the genera considering that the entry of a pathogen like powdery mildew do not normally take place through stomata, this relation was not expected. Number of oilglands per unit area of leaf also showed no indication of having any positive correlation.

Table 4. Thickness of cuticle and epidermal layer of susceptible and resistant powdery mildew

Thickness (μm)							
Young				Old			
Upper Surface		Lower Surface		Upper Surface		Lower Surface	
Cuticle layer	Cuticle +Epidermal layer	Cuticle layer	Cuticle +Epidermal layer	Cuticle layer	Cuticle +Epidermal layer	Cuticle layer	Cuticle +Epidermal layer
DARJEELING ORANGE							
6.22 to 10 37	20 75 to 26.97	4.15 to 6.22	16 6 to 20 75	4.15	16.6 to 20.75	2.75 to 3.32	10 37 to 12.45
TRIFOLIATE							
4 15 to 8.3	18.67 to 24.9	4.15	16.5 to 20.75	4.15 to 6.22	12.45 to 18.67	4.15 to 6.22	14.52 to 20.75

The relation between powdery mildew disease with thickness of cuticle and epidermal layer of susceptible (Table 4) did not vary significantly. It was found that susceptible genera had more thick cuticle and epidermal layer than the resistant variety except the lower surface of the leaves. It may be explained that some unknown chemical present in the cutical which regulate the penetration pattern of the powdery mildew pathogen (Yearwood, 1957).

ACKNOWLEDGEMENTS

The authors are thankful to Hill Development Branch Secretariat, Govt. of West Bengal and Darjeeling Gorkha Hill Counell, Govt. of West Bengal for providing the financial support under the project "Citrus Die-back Mapping and Control Project" during the tenure of which this work was carried out.

REFERENCES

Devarajan, M. R. (1946). Powdery mildew of orange in Coorg. *Indian Farming* 7 (6): 303-304.

Dutta Roy, S. (1990). Fungal disease problem of mandarin orange (*Citrus reticulata* Blanco) in Darjeeling district: Powdery mildew. Ph. D. thesis. Bidhan Chandra Krishi Viswadidyalaya.

Dutta Roy. S. Mukherjee, N. and Mukhopadhyay, S. (1986). Disease problem of mandarin orange in Darjeeling district. Fungal disease. Proceeding of the 4th National *Citrus* seminer. pp. 99-104.

Narayanappa, M. and Ravishankar, H. (1985). A note on the field evaluation of *Citrus* scion varieties on the incidence of powdery mildew. *Haryana Journal of Horticultural Science* **14** (1/1): 61-64.

Narayanappa, M. and Ravishankar, H. (1986). Reaction of root stock varieties of *Citrus* to powdery mildew under field condition. *Journal of Maharashtra Agricultural University* **11** (1): 111-112.

Raghavendra Rao, N. N., Ram, B., Naidu, R. and Rao, D. G. (1977). Fungal disease of citrus and their control. International symposium of citriculture pp. 42 (Abs.).

Ram, B., Naidu, R., Sohi, H. S. and Ullasa, B. A. (1977). Fungal diseases of mandarin in Malanard region and their control. International symposium of citriculture pp. 42 (Abs.).

Sass, J. E. (1951). Botanical microtechnique. Oxford and J. B. H. Publishing Co., Calcutta, India.

Ullasa, B. A. and Naidu, R. (1975). Field screening of *Citrus* varieties against powdery mildew (*Acrosporinum tingtaninum* (Carter) Subram). *Indian Journal of Horticulture* **32** (2 and 4): 156-159.

Yearwood, C. E. (1957). Powdery mildews. *The botanical review*. **23** (4) : 235-293.

(Accepted for publication 1 April 1991)